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# The Identification of Polychlorinated Terphenyls at Trace Levels in Human Adipose Tissue by Gas Chromatography/Mass Spectrometry

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EPA-600/J-78-044
JOURNAL ARTICLE

# The Identification of Polychlorinated Terphenyls at Trace Levels in Human Adipose Tissue by Gas Chromatography/Mass Spectrometry

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## Abstract

**Pooled samples of human adipose extracts representative of the general population of the United States were examined by gas chromatography-mass spectrometry. The use of gel permeation chromatography for cleanup was found to offer a significant improvement in detection capability. In addition to previously documented organochlorine residues, numerous polychlorinated terphenyls and three additional components of technical chlordane were identified.**

## Introduction

For several years, a program of routine examination of human adipose tissue samples for organic pollutants has been carried out by the U.S. Environmental Protection Agency (EPA) under the National Human Monitoring Program (1). Individual autopsy and surgical samples collected throughout the United States are analyzed by contractor laboratories, which use gas chromatography for identification and quantification. Periodically pooled sample extracts are submitted to the EPA for residue confirmation by combined gas chromatography-mass spectrometry (GC/MS).

These extracts are derived from a modified Mills-Onley-Gaither (MOG) cleanup procedure (2) and present several obstacles to GC/MS analysis as a result of residual lipid material. Concentration of the extracts to the levels required for GC/MS identification of low level components is greatly hindered by solidification of the material. Large amounts of fatty substances also lead to a rapid loss of mass spectrometer sensitivity due to contamination of the ion source and quadrupole analyzer. Deterioration of chromatographic columns under these conditions is also frequently encountered. Furthermore, the elution of lipids and their degradation products from the GC column presents a substantial MS background.

Gel permeation chromatography (GPC), introduced by Stalling and co-workers (3) and developed into an automated system by Tindle and Stalling (4), has been reported to yield high recoveries of many pesticides while removing ca. 98% of the fatty material from sample extracts. Cleanup using acetonitrile partitioning was found to be less efficient than GPC (5). Recoveries of several chlorinated pesticides from GPC were reported (6) to be comparable with those obtained with the Pesticide Analytical Manual procedure (7). Preliminary experiments in our laboratories have demonstrated the feasibility of applying GPC cleanup to the analyses of human adipose extracts (8).

In this report we communicate the first application of GPC to the cleanup of human tissue extracts for GC/MS analysis. In addition, we report the first findings of polychlorinated terphenyls (PCTs) as apparently ubiquitous contaminants in the U.S. population.

## Experimental

*Materials and Standards.* All analytical standards except Aroclor® 5460 were obtained from the Pesticide Repository, U.S. Environmental Protection Agency, Research Triangle Park, NC. Aroclor 5460 was supplied by RFR Corp. (Hope, RI).

All solvents used were Distilled-in-Glass™ (Burdick and Jackson, Muskegon, MI) or equivalent.

*Samples.* One composite sample of human adipose tissue extract was obtained directly from each of the two contract laboratories participating in the National Human Monitoring Program. These samples represented the pooled extracts of 209 and 245 individual tissue samples collected from all nine of the census regions of the conterminous United States and corresponded to total wet tissue weights of 647 g and 822 g, respectively. Each individual extract had been subjected to the MOG cleanup procedure and the 6% diethyl ether in petroleum ether fraction from Florisil chromatography selected for pooling.

*Gel Permeation Chromatography.* GPC cleanup was performed on an Analytical Biochemistry Laboratories Autoprep® Model 1001 gel permeation chromatograph equipped with a 35-cm x 25-mm (i.d.) glass column containing 200-400 mesh SX-3 Bio-Beads® (Bio-Rad Laboratories, Richmond, CA). The elution volumes and separation characteristics of the GPC column in use were established with a pesticide-fortified fat extract. One half of each composite sample was taken for cleanup. The aliquots were evaporated to near dryness with a nitrogen stream and then diluted to 8 mL with toluene in ethyl acetate (1:3, v/v). The total volume was introduced into the instrument, which utilized a 5-mL aliquot for cleanup. Toluene in ethyl acetate (1:3, v/v) was used for elution at a flow of ca. 5 mL/min. The initial eluent (ca. 100 mL), which contained fatty substances, was discarded after the absence of organochlorine compounds was confirmed by electron capture GC. The subsequent fraction (ca. 90 mL), which contained pesticide residues, was collected and concentrated

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in a rotary evaporator to ca. 10 mL. Further volume adjustments were accomplished under a nitrogen stream. With this method, the average recovery of standards was 90% or greater. Reagent blanks showed no detectable pesticide or chlorinated organic residues.

*Gas Chromatography/Mass Spectrometry.* All analyses in this study were performed on a Finnigan 3200 mass spectrometer equipped with a Finnigan 9500 gas chromatograph and a Finnigan 6100 data system. Electron impact ionization was utilized with 70 eV electron energy and 0.5 mA emission current. The separator oven and transfer line were maintained at 250°C. Helium was used as the carrier gas at a flow of 20 mL/min. The GC inlet temperature was 225°C.

Prior to GPC the composite samples were concentrated under a stream of nitrogen until they became viscous (at ca. 2 mL). About 7 μL of each solution was subjected to GC/MS analysis. Following GPC cleanup, samples were concentrated to 1 mL to obtain GC/MS comparison data at a concentration approximating that of the untreated sample. The samples were then further concentrated to volumes appropriate for trace determinations.

Initial surveys were made on a 152-cm x 2-mm (i.d.) glass column packed with 1.5% OV-17/1.95% OV-210 on 80/100 mesh Gas Chrom ® Q. Temperature-programmed analyses were made from 150° (1 min) to 275°C at 2°C/min. Spectral data were acquired over the range 100 to 550 amu at ca. 6 sec/scan.

GC/MS analyses to confirm the polychlorinated terphenyl residues were made using a 152-cm x 2-mm (i.d.) glass column packed with 3% OV-1 on Gas Chrom Q. The column oven was programmed from 150°C (1 min) to 300°C at 4°C/min. Mass spectra were acquired over the range 420 to 720 amu at 6 sec/scan for PCT confirmation and over the range 100 to 600 amu at ca. 5 sec/scan to compare the untreated and GPC-cleaned samples.

## Results and Discussion

Two different samples representing composites of the 6% fractions from the Florisil cleanup (2) of human adipose tissue were examined by GC/MS before and after GPC cleanup. Certain advantages of the gel fractionation procedure were immediately obvious. Whereas concentration of the original samples was limited to a final volume of ca. 2 mL by the viscosity of the material, the samples treated by GPC could be concentrated 10-fold further without difficulty. Figure 1 compares total ion current reconstructed gas chromatograms (RGCs) obtained from equivalent injections before and after GPC treatment.

A detailed examination of the GC/MS data acquired from the GPC-cleaned extracts confirmed the presence of hexachlorobenzene; β-BHC (1e,2e,3e,4e,5e,6e-hexachlorocyclohexane); p,p'-DDT along with its metabolite p,p'-DDE; and the chlordane/heptachlor residues oxychlordane, heptachlor epoxide, and "early"-, *cis*-, and *trans*-nonachlor (c.f. Ref. 13 for a discussion of chlordane components). Twenty-eight chromatographically resolvable polychlorinated biphenyls, ranging from di- to decachloro, were also identified. Most of these residues have been previously identified in human adipose by GC/MS (9-12). Small quantities of intact α- and γ-chlordane, usually metabolized to oxychlordane in humans (10,11), were identified. Another compound with a mass spectrum (characterized by an eight-chlorine isotope cluster beginning at 419 amu) and a GC retention time closely matching those of a minor component of technical chlordane (GC peak 23, ref. 13) was also detected.

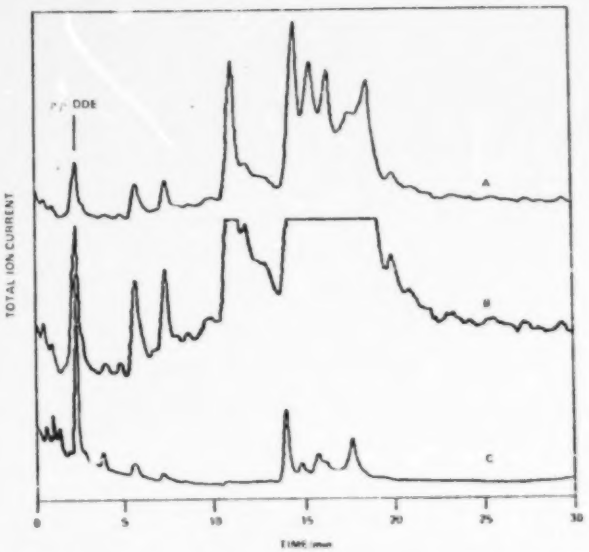


Figure 1. Reconstructed total ion chromatograms (m/e 100 through 600, OV-1 column) of human adipose sample: (a) Prior to GPC cleanup, normalized on the major peak; (b) Prior to GPC cleanup, normalized on p,p'-DDE; (c) After GPC cleanup.

During the GC/MS investigation of one of the GPC-cleaned extracts, data collection was routinely terminated following the elution of decachlorobiphenyl. However, periodic monitoring of the mass spectrum of the GC effluent was continued in order to determine when a second sample analysis might be feasible. During the course of this monitoring, several late-eluting components were observed that appeared to yield high mass fragments with isotope clusters typical of polychlorinated compounds. The masses observed compared favorably with those expected for polychlorinated terphenyls (PCTs, cf. Figure 2) (14). The bandwidths of these late-eluting components were such that the acquisition of quality mass spectra was not practical. However, it was possible to confirm the presence of numerous PCTs in both pooled samples by GC/MS with a 3% OV-1 column. Table 1 summarizes these findings. A mass spectrum typical of those obtained during

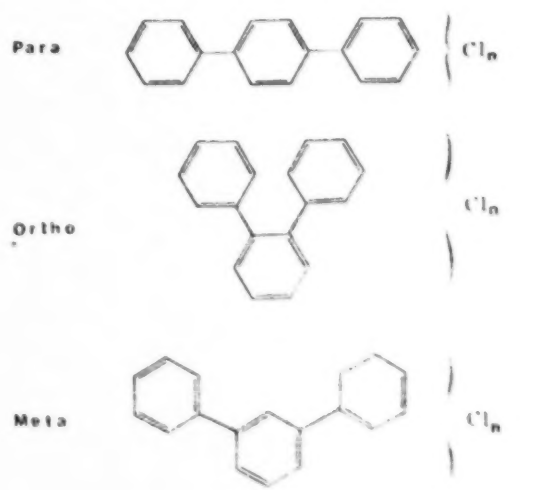


Figure 2. Possible structures of polychlorinated terphenyls.

**Table I. Polychlorinated Terphenyls Indentified in Human Adipose Samples**

| Number of Chlorine Atoms | Number of Chromatographically Resolved Isomers |
|---|---|
| 7 | 2 |
| 8 | 8 |
| 9 | 5 |
| 10 | 5 |
| 11 | 3 |

the confirmation analyses is presented in Figure 3 along with pertinent mass assignments.

The distribution of PCT isomers identified in this study resembles that found in Aroclor 5460, a technical mixture of PCTs. Figures 4 and 5 compare the limited mass RGCs obtained for one of the composite samples with the corresponding chromatograms for Aroclor 5460. The mass ranges covered include the major ions in the isotope clusters for the molecular ions of the indicated terphenyls. In each case, however, some interference may be anticipated from the $[M\text{-}Cl_2]^+$ fragment ions of homologs containing two additional chlorines. Both the heptachloro- and octachloroterphenyl traces show the influence of the $[M\text{-}Cl_2]^+$ fragments of nonachloro- and decachloroterphenyls, respectively. Comparison of Figures 4a and b with 4c and d indicates that the $[M\text{-}Cl_2]^+$ fragments from the nonachloroterphenyls are major contributors to the latter chromatograms. This observation is in qualitative agreement with the Aroclor 5460 GC/MS analysis by Putnam et al. (14), which implies a greater concentration of nonachloro isomers. In contrast to their results, no penta- or hexachloroterphenyls were detected in this study in either the standard or the biological samples. This discrepancy may be due to differences in the origins of the standards or to sample size.

A comparison of Figures 1 and 4 clearly illustrates the interference with PCT determination caused by lipid materials and the advantages of GPC cleanup for this purpose. The PCTs identified in this study were present in minute quantities and could be detected only at the concentration levels obtainable with GPC cleanup. In addition to the two composite tissue extracts examined, PCTs were confirmed by direct probe MS in two of three individual human adipose samples, which were extracted and cleaned up in this

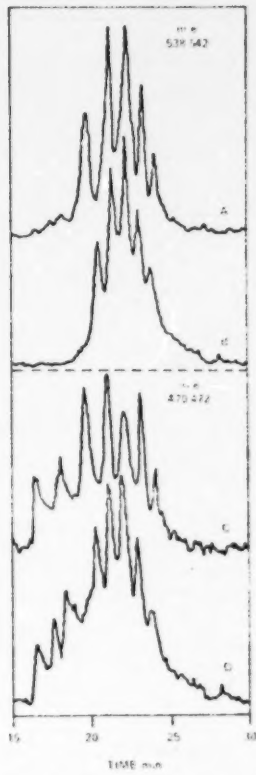

Figure 4. Limited mass reconstructed gas chromatograms comparing 9-chlorine PCTs in human adipose (a) and in Aroclor 5460 (b) and 7-chlorine PCTs in human adipose (c) and Aroclor 5460 (d).

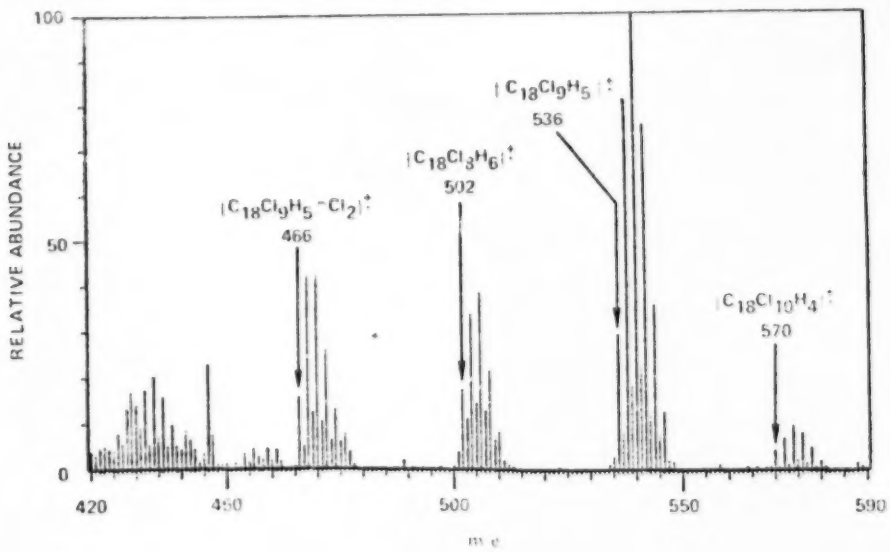


Figure 3. Mass spectrum confirming 8-, 9-, and 10-chlorine PCTs in human adipose sample.

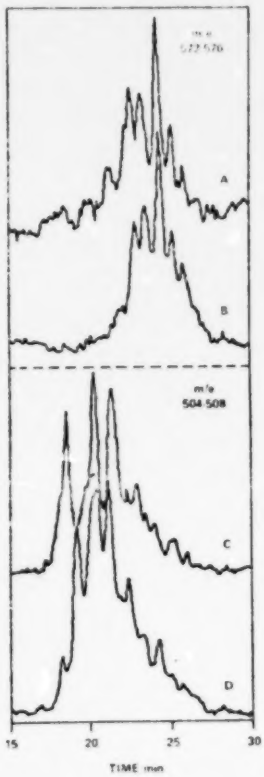


Figure 5. Limited mass reconstructed gas chromatograms comparing 10-chlorine PCTs in human adipose (a) and in Aroclor 5460 (b) and 8-chlorine PCTs in human adipose (c) and Aroclor 5460 (d).

laboratory by the combined MOG-GPC technique.

Information regarding environmental contamination with PCTs has begun to accumulate over the past few years. Japanese and European investigators have reported PCT residues in a variety of samples, including marine life (15), avian tissue (16), and human fluids and tissues (16, 17). Canadian efforts have demonstrated the presence of PCTs in food, food packaging materials (18), and in avian samples (19). Within the United States, PCT accumulations have been reported near user facilities (20), and the possibility of their introduction into human food supply has been investigated (21). The findings reported here indicate that an accumulatin of PCT residues within the general population of the United States has occurred.

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